

## CLAIMS

What is claimed is:

1. A method for detecting a nucleic acid target, the method comprising:
  - 5 a) hybridizing a nucleic acid target, comprising a target nucleic acid sequence, to a nucleic acid probe, comprising a probe nucleic acid sequence, wherein the target comprises a binding ligand;
  - b) contacting the hybridized target with a receptor comprising multiple sites capable of binding the binding ligand to complex the receptor to the binding ligand;
  - 10 c) contacting the receptor with an amplification reagent, comprising a plurality of the binding ligands, to complex the amplification reagent to the receptor; and
  - d) detecting the presence of the complexed amplification reagent.
- 15 2. The method of claim 1, wherein the amplification reagent comprises an antibody.
3. The method of claim 1, wherein the amplification reagent comprises a DNA matrix.
- 20 4. The method of claim 3, wherein the DNA matrix comprises subunits of partially double stranded and partially single stranded DNA molecules.
- 25 5. The method of claim 3, wherein the DNA matrix comprises:  
a plurality of molecules of a first partially double stranded polynucleotide, the polynucleotide having a first molecule end, a second molecule end and a double stranded body portion intermediate of the first and second ends, wherein the first and second ends each comprise at least one of first and second arms consisting of a single strand of polynucleotide, and wherein the single strands are hybridizable with a predetermined nucleic acid sequence of nucleotides in a nucleic acid, and the first and second arms of each of said first and second ends are nonhybridizable with each other;
- 30 a plurality of molecules of a second partially double stranded polynucleotide, each polynucleotide including a first molecule end, a second molecule end and a double stranded body portion intermediate the first and second

5 ends, the first and second ends each having at least one of first and second arms consisting of a single strand of polynucleotide that is hybridizable with a predetermined nucleic acid sequence of nucleotides in a nucleic acid, wherein the first and second arms of each of said first and second ends are non-hybridizable with each other;

10 wherein the plurality of molecules of the first polynucleotide and the second polynucleotide are joined together through annealing of one or more arms thereof, to form a matrix, and wherein arms of the plurality of first and second polynucleotide molecules located on the outer surface of the matrix are hybridized to nucleic acids having the binding ligand attached thereto.

15 6. The method of claim 1 wherein at least one of the receptor and the amplification reagent comprises a detectable label; and

20 wherein step d) comprises detecting the label.

25 7. The method of claim 1 further comprising labeling at least one of the receptor and the amplification reagent with a detectable label prior to step d); and

30 wherein step d) comprises detecting the label.

35 8. The method of claim 1 wherein the method further comprises, after step c), and before step d), the step of contacting the amplification reagent, comprising a plurality of the binding ligands, with labeled receptor molecules thereby to complex a plurality of labeled receptor molecules to the amplification reagent; and

40 wherein step d) comprises detecting the labeled receptor molecules complexed to the amplification reagent.

45 9. The method of claim 8, wherein the label is selected from the group consisting of a fluorescent label, a gold label and an enzyme label.

50 10. The method of claim 9, wherein the fluorescent label is selected from the group consisting of fluorescein, rhodamine, resorufin, and a coumarin.

55 11. The method of claim 1, wherein the binding ligand comprises biotin and the receptor comprises avidin or streptavidin.

60 12. The method of claim 1, wherein the amplification reagent comprises an antibody capable of specifically binding the receptor.

13. The method of claim 12, wherein the binding ligand comprises biotin, the receptor comprises avidin or streptavidin, and wherein biotin is covalently attached to the antibody.

5 14. The method of claim 13, wherein the receptor comprises streptavidin and the antibody is an anti-streptavidin antibody comprising a plurality of biotin molecules covalently attached to the antibody;

10 wherein the method further comprises, after step c), and before step d), the step of contacting the antibody with labeled streptavidin, thereby to complex a plurality of labeled streptavidin molecules to the antibody; and

15 wherein step d) comprises detecting the labeled streptavidin molecules complexed to the antibody.

15. The method of claim 11, wherein the amplification reagent comprises a DNA matrix comprising single stranded DNA; and

20 wherein biotin is attached to the DNA matrix by hybridization of a plurality of biotinylated nucleic acids to single strands of the DNA matrix.

25 16. The method of claim 15, wherein the method further comprises, after step c), and before step d), the step of contacting the DNA matrix with labeled streptavidin, thereby to complex a plurality of labeled streptavidin molecules to the DNA matrix; and

20 wherein step d) comprises detecting the labeled streptavidin molecules complexed to the DNA matrix.

17. The method of claim 16, wherein the label is selected from the group consisting of a fluorescent label, a gold label and an enzyme label.

25 18. The method of claim 17, wherein the fluorescent label is selected from the group consisting of fluorescein, rhodamine, resorufin, and a coumarin.

19. The method of claim 1, wherein the nucleic acid probe is immobilized on a surface.

30 20. The method of claim 19, wherein the surface is selected from the group consisting of Langmuir Blodgett film, glass, germanium, silicon, (poly)tetrafluorethylene, polystyrene, gallium arsenide, gallium phosphide, silicon oxide, silicon nitride, and combinations thereof.

21. The method of claim 5 wherein, in step (a), the hybridization is conducted in a hybridization solution comprising a sulfonate buffer.

22. The method of claim 14 wherein, in step (a), the hybridization is conducted in a hybridization solution comprising a sulfonate buffer.

23. A method for detecting a nucleic acid target, the method comprising:

- 5 a) providing a substrate comprising a surface, the surface comprising at least 100 nucleic acid probes, each nucleic acid probe contained in an area of less than about 0.1 cm<sup>2</sup>, and each nucleic acid probe having a defined sequence and location on the surface;
- 10 b) contacting the surface with a nucleic acid target, comprising a target nucleic acid sequence, to permit the nucleic acid target to hybridize with at least one selected nucleic acid probe that comprises a probe nucleic acid sequence capable of hybridizing to the target nucleic acid sequence, and wherein the target comprises a binding ligand;
- 15 c) contacting the hybridized target with a receptor comprising multiple sites capable of binding the binding ligand to complex the receptor to the binding ligand;
- 20 d) contacting the receptor with an amplification reagent, comprising a plurality of the binding ligands, to complex the amplification reagent to the receptor; and
- 25 e) detecting the presence of the complexed amplification reagent.

24. The method of claim 23 wherein, in step b), the surface is contacted with the nucleic acid target in a hybridization solution comprising a sulfonate buffer.

25. The method of claim 24 wherein the sulfonate buffer is 2-[N-morpholino]ethanesulfonic acid ("MES").

26. The method of claim 23, wherein the amplification reagent comprises a DNA matrix, the binding ligand comprises biotin and the receptor comprises streptavidin.

27. The method of claim 26, wherein the binding ligand comprises biotin, the receptor comprises streptavidin and the amplification reagent comprises an anti-streptavidin antibody.

30 28. A complex comprising:  
a nucleic acid comprising a binding ligand;

a receptor comprising multiple binding sites capable of binding the binding ligand; and

an amplification reagent comprising a plurality of said binding ligands.

5 29. The complex of claim 28, wherein the binding ligand comprises biotin and the receptor comprises streptavidin or avidin.

30. The complex of claim 29, wherein the amplification reagent comprises a DNA matrix.

10 31. The complex of claim 29, wherein the amplification reagent comprises an anti-streptavidin antibody.

32. A substrate comprising a surface having immobilized thereon a nucleic acid probe, comprising a probe nucleic acid sequence, hybridized to a nucleic acid target, comprising a target nucleic acid sequence;

15 wherein the target comprises a binding ligand, and wherein the binding ligand on the target is complexed with a receptor comprising multiple sites capable of binding the binding ligand, and wherein the receptor is complexed to an amplification reagent, comprising a plurality of the binding ligands.

33. The substrate of claim 32, wherein the binding ligand comprises biotin and the receptor comprises streptavidin or avidin.

20 34. The substrate of claim 33, wherein the amplification reagent comprises a DNA matrix.

35. The substrate of claim 33, wherein the amplification reagent comprises an anti-streptavidin antibody.